'AD_____

GRANT NUMBER DAMD17-96-1-6287

TITLE: Gene Therapy Mediated Breast Cancer Immunity

PRINCIPAL INVESTIGATOR: Stephen Eck, M.D., Ph.D.
                        Heike Boxhorn

CONTRACTING ORGANIZATION: University of Pennsylvania
                          Philadelphia, PA   19104

REPORT DATE: September 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
              U.S. Army Medical Research and Materiel Command
              Fort Detrick, Frederick, Maryland   21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
                        distribution unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

1

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE September 1998	3. REPORT TYPE AND DATES COVERED Annual (15 Aug 97 – 14 Aug 98)

4. TITLE AND SUBTITLE
Gene Therapy Mediated Breast Cancer Immunity

5. FUNDING NUMBERS
DAMD17-96-1-6287

6. AUTHOR(S)
Stephen Eck, M.D., Ph.D.
Heike Boxhorn

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pennsylvania
Philadelphia, PA 19104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

19981118 044

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

In an effort to determine why B7-1 expressing breast cancer cells fail to stimulate T cells to proliferate, we sought to characterize the immune inhibitory factors secreted by breast cancer cells. We have previously shown that some human breast cancer cell lines produce factors that inhibit the proliferation of activated T cells. Here, we tested a larger pool of breast cancer and mammary carcinoma cells and found that ten of fifteen breast cancer cells block thymidine uptake of activated lymphocytes. Most of these breast cancer cell lines produce high amounts of prostaglandin E_2 (PGE_2). Using PGE_2 depletion studies we demonstrated for the MCF-7 cell line that PGE_2 accounts for most of the immunosuppressive effect in vitro. Among four tested mammary carcinoma cell lines three secreted PGE_2 by the activity of cyclooxygenase 2 (COX-2) as detected by Northern blot analysis. In summary, PGE_2 secretion by breast cancer cells appears to impair the efficiency of B7-1 modified breast cancer vaccines.

14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 20
adenovirus, B7, T cell, immunity, gene therapy			16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

__X__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

_____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

__X__ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

_____ Herbe Boston _____ 8/26/98
PI - Signature   Date

Table of contents

Introduction

Breast carcinoma cells produce high levels of immunosuppressive factors including prostaglandin E_2 (PGE_2, (1), all of which have been shown to affect anti-tumor immunity on several levels. PGE_2 has been shown to inhibit differentiation of lymphokine activated killer cells (LAK), suppression of natural killer cell (NK) activity (2, 3, 4) and downregulation of a humoral response (5). Furthermore, PGE_2 has been shown to inhibit T cell proliferation in lymphocyte cultures through the downregulation of MHC class II expression on antigen presenting cells (6, 7, 8) and through suppression of cytokine production (9, 10).

PGE_2 is produced by COX mediated oxidation of arachidonic acid and has been found in some human breast cancer cell lines. There are two isoforms of COX, designated COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and mediates the synthesis of prostaglandins required for normal physiological functions, such as maintaining gastrointestinal, kidney and reproductive functions (11). COX-2 is not detectable in normal tissue but is inducible by cytokines, growth factors, oncogenes and tumor promoters (12). COX-2 activity is upregulated in breast cancer (13) and associated with elevated levels of PGE_2 in breast carcinoma in vivo and with tumors that exhibit a high metastatic potential (13, 14). To study the contribution of PGE_2 to tumor development and progression, a strategy that eliminates PGE_2 synthesis in vivo is needed. Due to the complexity of the biologic functions of PGE_2, complete inhibition of prostaglandin synthesis in all tissues is not a rationale goal. Rather than inhibiting prostaglandin synthesis nonspecifically, the blockage of tumor-specific PGE_2 by a selective COX-2 inhibitor is preferable. Several drugs that specifically inhibit COX-2 have been studied in a preventive and adjuvant setting (15, 16). Targeted inhibition of COX-2 or the combination of selective COX-2 inhibition and a compound that suppresses COX-2 expression are underway (17).

We have previously shown that human B7-1 expressing MCF-7 cells were not able to stimulate allogeneic T cells to proliferate under conditions where B7-1 transduced melanoma cells stimulated T cell proliferation. The failure of B7-1 expressing MCF-7 cells to induce T cell proliferation was due in part to soluble immunosuppressive agents produced by the tumor cells. Here, we provide evidence that tumor-derived PGE_2 is at least in part responsible for curtailing T cell proliferation in this experimental setting.

Materials and Methods

Cell lines and culture conditions

The human metastatic melanoma cell line WM9 (provided by Dr. M. Herlyn, Wistar Institute, Philadelphia, PA) was cultured in MCDB-153 base medium (Sigma, St. Louis, MO) containing 20% L-15, 2% FBS (Gibco, Grand Island, NY) and 0.01 mg/ml insulin (Sigma). The human breast cancer cell lines SUM52PE, SUM149PT, SUM185PE and SUM190PT were described earlier (18) and were cultured in Ham's F-12 medium containing 5% FBS, insulin (5 μg/ml) and hydrocortisone (1 μg/ml). All other human breast cancer cell lines and the human normal breast epithelial cell line HBL-100 were obtained from the American Type Culture Collection (ATCC, Rockville, MD). MCF-7 cells were cultured in DMEM medium containing 10 % FBS, 1% penicillin/streptomycin and 10 μg/ml insulin. MCF-10 and MDA MB 231 cells were cultured in 50% DMEM medium, 50% F-12 medium supplemented with epidermal growth factor (20 ng/ml), cholera toxin (0.1 μg/ml), insulin (2 μg/ml) and hydrocortisone (0.5 μg/ml). The growth medium of BT-20 cells was DMEM medium, 10% FBS, 1% penicillin/streptomycin and insulin (2 μg/ml). BT-474 cells were cultured in L-15 medium (Sigma), 10% FBS, 1% penicillin/streptomycin. SK-BR-3 and T47-D cells were cultured in Mc Coy's 5a medium, 10 % FBS and RPMI-medium, insulin (2 μg/ml) and 10% FBS, respectively. The mammary carcinoma cell line T2994 was provided by Dr. W. M. F. Lee (University of Pennsylvania, PA). The mammary carcinoma cell line MT901 was provided by Dr. L. A. Turka and the mammary carcinoma SCK cells were provided by Dr. G. Rhee (University of Maryland, MD). The T2994 cells were grown in F-12 medium, containing 20% calf serum, gentamycin (Gibco, 50 μg/ml), insulin (5 μg/ml) and epidermal growth factor (10 μg/ml). MT-901 cells were cultured in RPMI medium supplemented with 10% FBS, 1% penicillin/streptomycin, gentamycin (50 μg/ml), sodium pyruvate (0.2 mM), L-glutamine (2 mM), non-essential amino acids (0.1 mM) and mercaptoethanol (50 μM). SCK cells were cultured in RPMI medium, 10% FBS, 1% penicillin/streptomycin.

Adenoviral transduction

Recombinant adenoviruses containing either the β-galactosidase reporter gene (Ad.lacZ), or the human B7-1 gene (Ad.hB7-1) were constructed as previously described (19, 20). WM9 cells (3×10^6 cells) were transduced in vitro with Ad.hB7-1 or Ad.lacZ at a multiplicity of infection (MOI) of 100 plaque forming units (pfu) per cell in growth medium containing 2% FBS. The next day, fresh growth medium was added and transgene expression was assessed by flow cytometry three days after transduction.

Flow cytometry

The expression of B7-1 before and after adenoviral transduction was detected using the BB-1/B7-1 mAb (Becton-Dickinson, Sunnyvale, CA) followed by a fluorescein isothiocyanate (FITC) labeled goat anti-mouse secondary antibody (Pharmingen, San Diego, CA). Ad.lacZ transduced tumor cells were incubated with fluorescein di-β-D-galactopyranoside (FDG, Molecular Probes, Eugene, OR) for one minute at 37°C followed by incubation on ice for 30 minutes. Cellular fluorescence (10,000 live cells/condition) were analyzed using a FACScan flow cytometer (Becton Dickinson). The purity of T cells was confirmed by incubation with an anti-CD3 antibody (Pharmingen) followed by a FITC-anti-mouse antibody (Pharmingen).

Production of media conditioned by tumor cells (CM)
For lymphocyte proliferation assays, conditioned medium from tumor cells ($3x10^6$ cells) cultured for 24 hours in 5 ml RPMI medium containing 10% heat inactivated FBS was collected. For the detection of PGE_2 by bioassays, MCF-7 cells were cultured under serum-free conditions for 24 hours (21). CM was stored in aliquots at -20°C (or at -80°C when used in bioassays for the detection of PGE_2).

Isolation of human peripheral blood mononuclear (MN) cells and purified T cells
Peripheral blood was collected in heparin tubes (Becton Dickinson, Franklin Lakes, NJ), diluted with an equal volume of PBS and underlaid with Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). After centrifugation at 3,000 rpm for 20 minutes at room temperature, the MN cell layer at the interface was removed, washed twice, and cell number was adjusted in RPMI medium, 10% heat inactivated FBS. Human T cells were isolated from the peripheral lymphocyte fraction by negative selection. Lymphocytes were incubated with an antibody mixture consisting of mouse mAbs to human CD14 to eliminate macrophages, CD16 to eliminate NK cells, CD19 to eliminate B cells and to MHC class II to eliminate MHC class II expressing cells (gift from Dr. L. A. Turka, University of Pennsylvania, PA) for one hour on ice. After washing, cells were resuspended in RPMI medium containing 2% FBS and incubated with BioMag® goat anti-mouse IgG magnetic beads (Perseptive Diagnostics, Cambridge, MA). After magnetic separation, the supernatant containing the T cell fraction was washed twice and counted. The functional purity of the T cell fraction was verified by their failure to proliferate in response to phytohemagglutinin (PHA) alone.

[^{3}H]-thymidine incorporation assay
WM9 cells ($1x10^6$) were treated with 100 µg mitomycin C (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 45 minutes at 37°C in serum-free RPMI medium. Cells were washed twice in RPMI medium and resuspended in RPMI medium containing 10% heat-inactivated FBS. Tumor cells ($2.5x10^4$ cells/well) and T cells or lymphocytes ($1x10^5$ cells/well) were co-cultured in 96-well round bottomed plates in RPMI medium containing 10% FBS. PHA (Boehringer Mannheim Biochemicals) was used at a concentration of 5 µg/ml. Phorbol myristate acetate (PMA, Calbiochem, La Jolla, CA) and ionomycin (Sigma) were added at a final concentration of 10 ng/ml and 360 ng/ml, respectively. After five days, cells were pulsed with one µCi [^{3}H]-thymidine (Dupont NEN, Boston, MA) and harvested 18 hours later. Thymidine incorporation was measured in cpm in a liquid scintillation counter (Wallac, Gaithersbury, MD). Each experiment consisting of quadruplicate samples was performed at least twice. Inhibition of [^{3}H]-thymidine incorporation was calculated as follows: % inhibition = 100% - (100% x cpm of PMA/ionomycin stimulated MN cells incubated with sample CM at a dilution of 1:4 / cpm of PMA/ionomycin stimulated MN cells).

Quantitative bioassay for PGE_2 and elimination of PGE_2 by affinity binding
The concentration of PGE_2 in the CM from different breast cancer cell lines was detected by a competitive enzyme linked immunoassay (EIA) kit (Cayman, Ann Arbor, MI) following the manufacturer's protocol. The column is designed for the specific purification of samples using a monoclonal anti-PGE_2 antibody covalently bound to sepharose with a loading capacity of 10 ng of PGE_2. PGE_2 standards were reconstituted in serum-free RPMI medium and samples were tested undiluted. The per cent standard

bound/maximum bound (%B/B$_0$) were calculated and plotted versus the PGE$_2$ concentration on a semi-log graph. The PGE$_2$ concentrations of the sample were determined by comparison to the standard curve. Untreated MCF-7 CM and PGE$_2$ depleted MCF-7 CM before and after the addition of exogenous PGE$_2$ were tested for their ability to inhibit the proliferation of stimulated MN cells. The depletion studies were performed twice.

Detection of COX-1 and COX-2 mRNA in mammary carcinoma cells by Northern blot analysis
To detect which isoform of the COX enzyme is present in the mammary carcinoma cells under investigation we extracted total mRNA from NT-5 cells, MT901 cells, T2994 and SCK cells using the Trizol (Gibco) method. Total RNA (20 µg per lane) was mixed with DMSO, glyoxal and NaPO$_4$ buffer and was fractionated on a 1.5 % agarose gel in 10mM NaPO$_4$ buffer. After electrotransfer to a 0.45 µ nylon membrane (Hybond, Amersham, IL), hybridization was performed using a^{32}P-labeled cDNA for COX-1 or COX-2 at 65°C overnight. Bands were visualized by exposure to an autoradiography film.

Results

PGE$_2$ mediates the inhibitory effect of MCF-7 CM on the proliferation of T cells.
Previous experiments showed that MCF-7 cells produced soluble factors that inhibit the proliferation of mitogen stimulated MN cells. We sought to determine whether PGE$_2$ is a potential breast tumor derived modulator of T cell proliferation. The secretion of PGE$_2$ by MCF-7 cells was tested by a competitive EIA. MCF-7 cells produced 40 pg/ml PGE$_2$ in a 24 h period. In order to determine whether MCF-7 derived PGE$_2$ accounted for the anti-proliferative effect, PGE$_2$ was removed from MCF-7 CM. We used an immunoaffinity column that specifically eliminates up to 10 ng PGE$_2$ (62 fold more than the loaded amount in the CM). The untreated MCF-7 CM and the PGE$_2$ depleted MCF-7 CM were tested for their inhibitory properties on the proliferation of PMA/ionomycin stimulated MN cells. MCF-7 CM inhibited the proliferative response of MN cells to PMA/ionomycin (Fig.1, circle). However, MCF-7 CM passed through this immunoaffinity column completely lost its inhibitory effect on PHA stimulated MN cells at all dilutions tested (Fig. 1, triangle). These results indicate that PGE$_2$ plays an important role in the inhibition of MN proliferation by MCF-7 CM.

Depletion of PGE$_2$ from the MCF-7 CM partially alleviates the proliferation of T cell stimulated with B7-1 expressing tumor cells
Previously, we showed that B7-1 expressing WM9 melanoma cells stimulated T cells to proliferate independently on PHA. These responses could be blocked by MCF-7 CM (Fig. 2A). Here, we investigated whether the removal of PGE$_2$ from MCF-7 CM restores the proliferation of T cells stimulated with B7-1 expressing melanoma cells. Untreated MCF-7 CM and MCF-7 CM that was PGE$_2$ depleted by affinity column was added to the co-culture of T cells and B7-1 expressing WM9 cells at a dilution of 1:4. When PGE$_2$ was depleted the proliferative response of T cells stimulated with B7-1 expressing tumor cells in PHA containing medium was partially alleviated (Fig. 2B). Some residual PGE$_2$ in MCF-7 CM that was not detectable by competitive EIA, could explain why the proliferative response was still blocked in the absence of PHA. In addition, when exogenous PGE$_2$ was added to the depleted MCF-7 CM, the proliferation of stimulated T cells was inhibited again (Fig. 2C). These results indicate that PGE$_2$ plays a role in the inhibitory effect of MCF-7 CM on T cell proliferation.

Breast cancer cells produce soluble factors that suppress T cell proliferation.
We sought to determine whether the inhibitory effect of MCF-7 CM was present in other breast cancer cell lines. We testes eleven human breast cancer cell lines and four mammary carcinoma cell lines. Eight of eleven human breast cancer cell lines tested (MCF-7, BT-20, MCF-10, BT-474, MDA-MB-231, SUM52PE, SUM149PT and SUM190PT) and 50% of the mammary carcinoma cell lines secreted soluble factors that were capable of inhibiting the proliferation of MN cells (Table 1). However, CM from SUM185 cells, T2294 cells, SK-Br-3 cells and the normal breast epithelial cells HBL-100 cells did not inhibit T cell proliferation under the same conditions. Thus, the production of soluble factors that inhibit lymphocyte proliferation is a common though not universal characteristic of breast cancer cells.

9

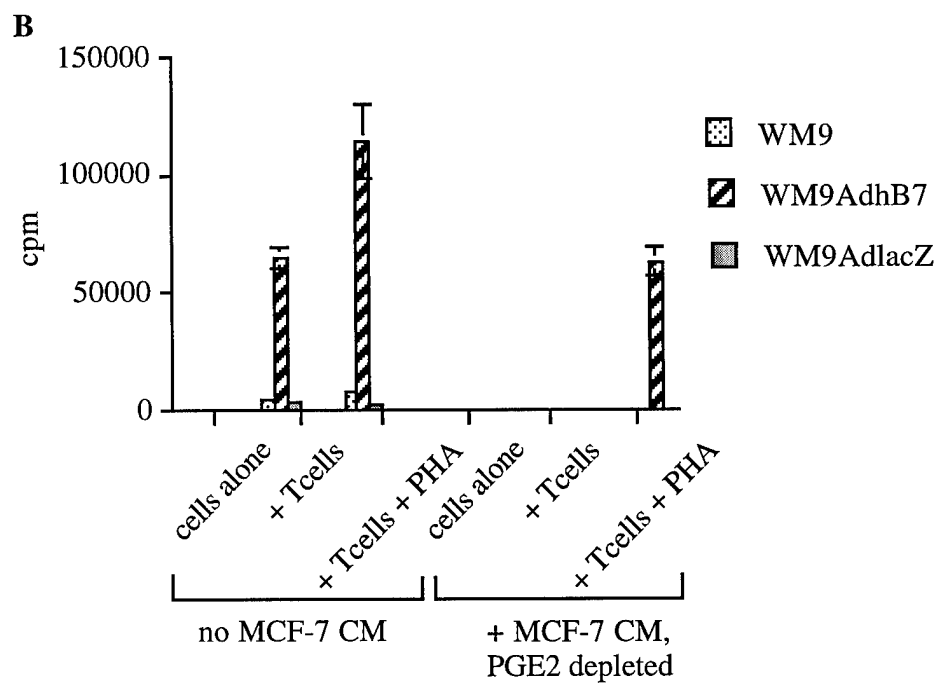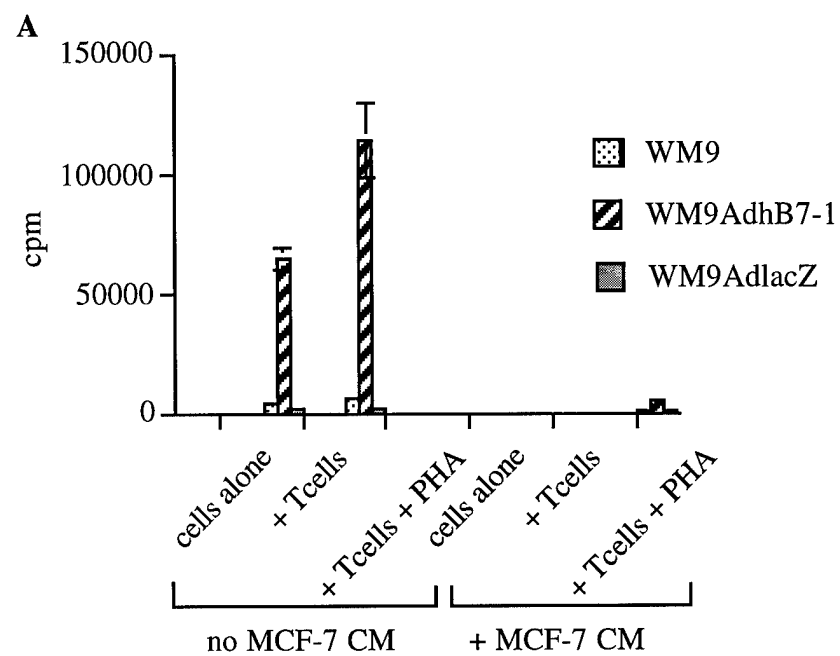**PGE$_2$ production by breast carcinoma cells.**

We next measured PGE$_2$ production by all breast cancer cell lines under investigation and by the normal breast epithelium cell line. We found that eleven of fourteen cell lines produced PGE$_2$ at detectable levels (Table 1). Interestingly, MCF-7 cells produced relatively low levels of PGE$_2$ (40 pg/ml) when compared to SUM190PT cells, SUM149PT cells, T2994 cells, MT901 cells and NT-5 cells which produce in excess of 1 ng/ml PGE$_2$. With the exception of HBL-100 and T2994 cells, all of the cell lines that produced PGE$_2$ also inhibited the proliferation of PHA stimulated MN cells although there was no correlation between the amount of secreted PGE$_2$ and the inhibitory capacity. In addition, BT-20 CM had a significant inhibitory effect although it did not produce detectable levels of PGE$_2$. Taken together, these results suggest that PGE$_2$ contributes to the inhibitory effect of breast cancer CM but may not be solely responsible for this effect.

Expression of COX mRNA in mammary carcinoma cells

It has been suggested that the overexpression and persistent expression of COX-2 in MDA-MB-231 cells is a feature of an aggressive phenotype (13). We tested four mammary carcinoma cell lines for their mRNA levels for COX-1 and COX-2 by Northern blot analysis. There was no detectable levels of COX-1 mRNA in the tested mammary carcinoma cell lines, whereas COX-2 mRNA was found in T2994 cells, MT901 cells and NT-5 cells. SCK cells were negative for COX-1 and COX-2 mRNA confirming that PGE$_2$ is not produced by these cells.
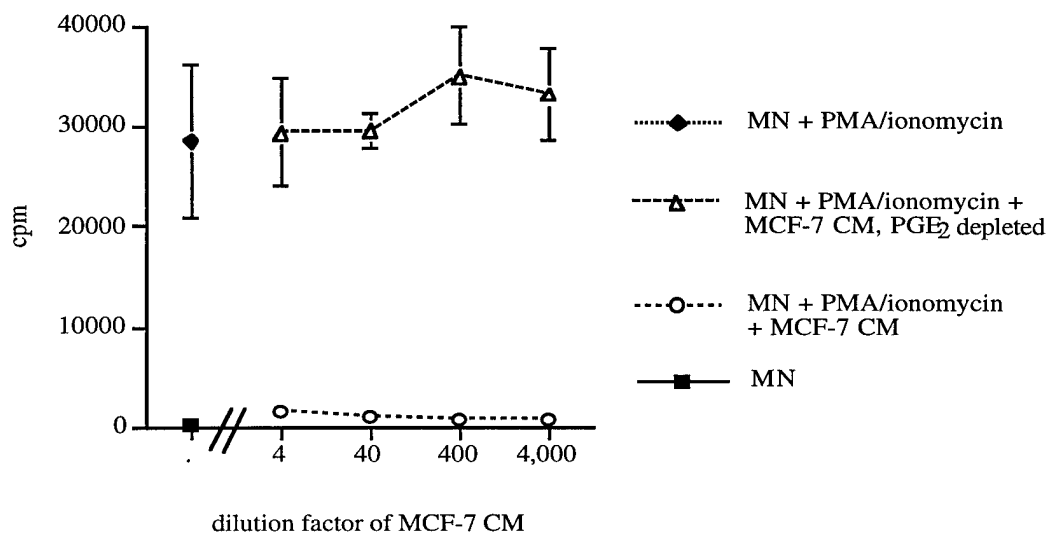
Fig. 1: The inhibitory effect of MCF-7 cell derived PGE_2 on the proliferation of MN cells stimulated with PMA/ionomycin. [^{3}H]thymidine incorporation by MN cells (square), by MN cells stimulated with PMA/ionomycin (diamond), by PMA/ionomycin stimulated MN cells incubated with MCF-7 CM (circle) and by PMA/ionomycin stimulated MN cells incubated with PGE_2-depleted MCF-7 CM (triangle). MCF-7 CM inhibited the mitogenic response of MN cells (circle). The inhibitory effect of MCF-7 CM was completely alleviated when PGE_2 was eliminated via PGE_2 affinity column (triangle).

A

WM9
WM9AdhB7-1
WM9AdlacZ

cells alone
+ Tcells
+ Tcells + PHA
cells alone
+ Tcells
+ Tcells + PHA

no MCF-7 CM
+ MCF-7 CM

B

WM9
WM9AdhB7
WM9AdlacZ

cells alone
+ Tcells
+ Tcells + PHA
cells alone
+ Tcells
+ Tcells + PHA

no MCF-7 CM
+ MCF-7 CM,
PGE2 depleted

C
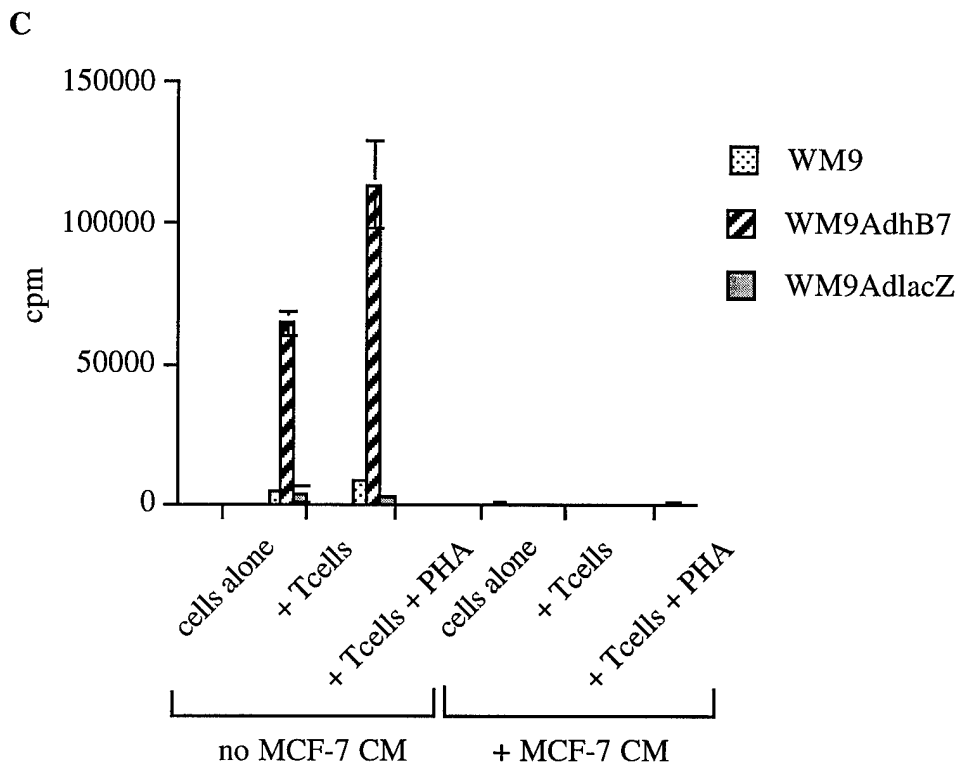
Fig. 2 : The effect of PGE_2 depleted MCF-7 CM on the proliferation of T cells stimulated with B7-1 expressing WM9 melanoma cells. The proliferation of T cells stimulated with B7-1 expressing WM9 cells (reflected by thymidine uptake in cpm) in the absence and presence of PHA was inhibited by the addition of MCF-7 CM (A). When PGE_2 was depleted from MCF-7 CM by affinity column, the proliferation of T cells incubated with B7-1 expressing WM9 cells and PHA was partially restored (B). The addition of exogenous PGE_2 to the depleted MCF-7 CM inhibited the proliferation of T cells again (C).

13
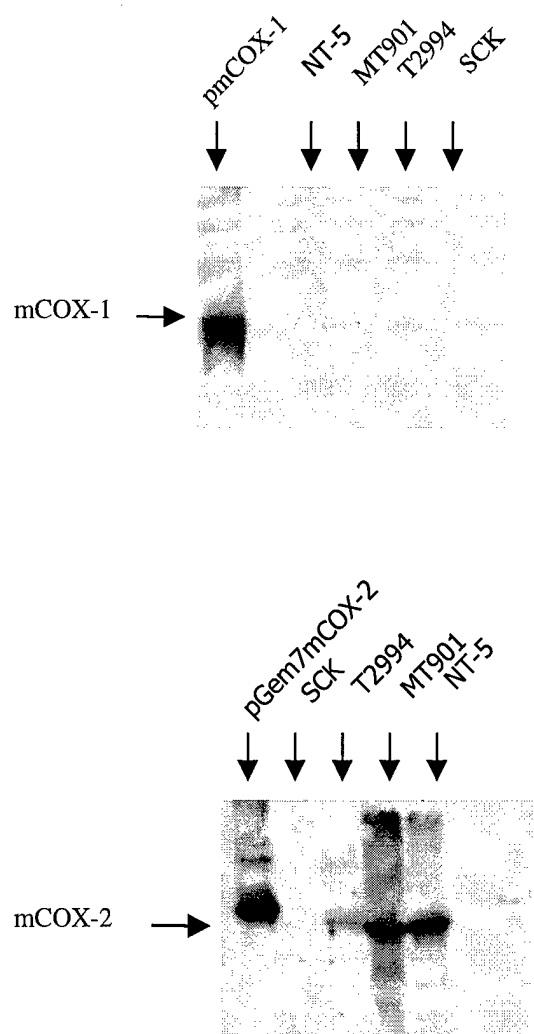
Fig. 3: Detection of COX-1 and COX-2mRNA in mammary carcinoma cells by Northern blot analysis.

human cell line	% inhibition of PHA stimulated MN cells	PGE$_2$ production (pg/ml)
HBL-100* (breast epithelial)	5%	100
BT-20	41%	0
MCF-10	99%	300
MCF-7	97%	40
BT-474	96%	40
MDA-MB-231	90%	260
SUM52PE	66%	30
SUM149PT	56%	>1000
SUM185PE	4%	0
SUM190PT	51%	>1000
T47D	1%	n.d.
SK-Br-3	1%	n.d.
SCK	1%	0
T2994	1%	1524
MT901	98%	1524
NT-5	90%	1156

Table 1: PGE$_2$ levels and inhibition of PHA stimulated MN cell proliferation by CM from a normal human breast epithelial cell line (HBL-100) and human breast carcinoma cell lines (all others).

Discussion

The present study demonstrates that the failure of B7-1 expressing breast cancer cells to stimulate T cell proliferation is due to the release of immunosuppressive factors by these tumor cells. We observed that MCF-7 CM when added to T cells stimulated with B7-1 expressing WM9 cells inhibited proliferation. PGE_2 was shown to be an important contributor to the T cell inhibitory effect of MCF-7 CM. Conclusive evidence for the involvement of PGE_2 in T cell growth inhibition was obtained when we removed PGE_2 from CM using an affinity column that specifically binds PGE_2. The selective elimination of PGE_2 from MCF-7 CM removed its MN cell growth inhibitory activity completely. Thus, inhibition of MN cell proliferation is mediated, at least in large part, by PGE_2 produced by MCF-7 cells. Furthermore, PGE_2 was detected in CM from additional breast cancer cell lines that also inhibited MN cell proliferation. The amount of PGE_2 production did not linearly correlate with inhibition of the proliferation of stimulated MN cells (Table 1). CM from the normal human breast epithelia cell line HBL-100 produced significant levels of PGE_2 (more than MCF-7) but did significantly suppress MN cell proliferation. Similarly, the CM from two cell lines (SUM149PT and SUM190PT) that produced the most PGE_2 showed only moderate inhibition of MN cell proliferation (Table 1). Although the minimal amount of PGE_2 needed to inhibit T cell proliferation has not been established, it appears likely that other factors in the breast cancer CM likely contribute to the effect. In support of this is the observation that BT-20 cells did not produce significant amounts of PGE_2 but inhibited PHA-dependent proliferation of MN cells by 41% (Table 1). This suggests that other tumor derived factors may induce immunosuppression as previously reported (22, 23). Nevertheless, ten out of thirteen breast cancer cells produced PGE_2, and all inhibited the proliferative response of MN cells to PHA. This, accompanied by the PGE_2 depletion data, suggests that PGE_2 contributes to tumor derived immunosuppression, although it is likely not the sole factor. To test the significance of PGE_2 in vivo, we want to express COX-1 or COX-2 in SCK cells. These cells do not express COX-1 or COX-2 and do ot make PGE_2. Construction of a PGE_2-secreting SCK cell line will allow direct comparison of PGE_2^+ with PGE_2^- mammary carcinoma cells with respect to their growth and immunogenicity. We also seek to reduce PGE_2 levels in MT901 cells and NT-5 cells by specific COX-2 inhibitors. In summary, this study provides evidence that PGE_2 derived from human breast cancer cells can contribute to an impaired anti-tumor immune response.

Recommendations in relation to the Statement of Work outlined in the proposal

We have previously shown that murine mammary carcinoma cells and human breast cancer cells can be efficiently transduced with Ad.mB7-1 and Ad.hB7-1, respectively (task 1 and 2 of the Statement of Work). We compared the in vivo growth behavior of untransduced and Ad.mB7-1 transduced mammary carcinoma cells in immunocompetent mice (task 5). Since there was no growth difference between B7-1 expressing mammary carcinoma cells and untransduced tumor cells we did not perform the immunogenicity studies proposed in task 7 and 8 but investigated an anti-tumor response in vitro. B7-1 expressing breast cancer cells failed to stimulate T cells to proliferate (task 9). Therefore, we hypothesized that human breast cancer cells produce immunoinhibitory factors. Thymidine incorporation assays revealed that the CM from most breast and mammary carcinoma cell lines inhibited T cell proliferation. Our data suggest that the tumor derived PGE_2 plays a role in T cell inhibition. The depletion of PGE_2 from MCF-7 CM abrogates the inhibitory effect on the proliferatin of mitogen stimulated lymphocyte proliferation (Task 9). We are currently characterizing the effect of breast cancer derived PGE_2 in vivo.

Conclusions

We have shown that breast cancer derived factors play a direct role in limiting a T cell mediated response against breast cancer cells. Evidence is provided that PGE_2 derived from breast cancer cells can contribute to the inhibition of cellular immunity in vitro. The production of PGE_2 can form, at least in part, a basis for the impaired anti-tumor immune response and has to be considered in immunotherapeutic strategies in breast cancer patients. Furthermore, specific strategies to circumvent the immunosuppressive effects of individual tumors may need to be devised for effective B7-1 mediated immunotherapy.

References

1.	Karmali, R. A., Welt, S., Thaler, H. T., and Lefevre, F. Prostaglandins in breast cancer: relationship to disease stage and hormone status, British Journal of Cancer. *48:* 689-96, 1983.

2.	Brunda, M. J., Herberman, R. B., and Holden, H. T. Inhibition of murine natural killer cell activity by prostaglandins, Journal of Immunology. *124:* 2682-7, 1980.

3.	Lala, P. K., Parhar, R. S., and Singh, P. Indomethacin therapy abrogates the prostaglandin-mediated suppression of natural killer activity in tumor-bearing mice and prevents tumor metastasis, Cellular Immunology. *99:* 108-18, 1986.

4.	Skibinski, G., Kelly, R., Harrison, C., McMillan, L., and James, K. Relative immunosuppressive activity of human seminal prostaglandins., Journal of Reproductive Immunology. *22:* 185-95, 1992.

5.	Miao, D., Skibinski, G., and James, K. The effects of human seminal plasma and PGE2 on mitogen induced proliferation and cytokine production of human splenic lymphocytes and peripheral blood mononuclear cells, Journal of Reproductive Immunology. *30:* 97-114, 1996.

6.	Valitutti, S., Castellino, F., Aiello, F. B., Ricci, R., Patrignani, P., and Musiani, P. The role of arachidonic acid metabolite PGE2 on T cell proliferative response, Journal of Clinical & Laboratory Immunology. *29:* 167-73, 1989.

7.	Laning, J. C., Isaacs, C. M., and Hardin-Young, J. Normal human keratinocytes inhibit the proliferation of unprimed T cells by TGFbeta and PGE2, but not IL-10, Cellular Immunology. *175:* 16-24, 1997.

8.	Arvind, P., Papavassiliou, E. D., Tsioulias, G. J., Qiao, L., Lovelace, C. I., Duceman, B., and Rigas, B. Prostaglandin E2 down-regulates the expression of HLA-DR antigen in human colon adenocarcinoma cell lines, Biochemistry. *34:* 5604-9, 1995.

9.	Murray, J. L., Dowd, J., and Hersh, E. M. In vitro inhibition of interleukin-2 production by peripheral blood lymphocytes from stage III melanoma patients by prostaglandin E2: enhancement of lymphocyte proliferation by exogenous interleukin-2 plus indomethacin, Journal of Biological Response Modifiers. *5:* 12-9, 1986.

10.	Vercammen, C. and Ceuppens, J. L. Prostaglandin E2 inhibits human T-cell proliferation after crosslinking of the CD3-Ti complex by directly affecting T cells at an early step of the activation process, Cellular Immunology. *104:* 24-36, 1987.

11.	Subbaramaiah, K., Zakim, D., Weksler, B. B., and Dannenberg, A. J. Inhibition of cyclooxygenase: a novel approach to cancer prevention, Proceedings of the Society for Experimental Biology & Medicine. *216:* 201-10, 1997.

12.	Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W., and Herschman, H. R. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue, Journal of Biological Chemistry. *266:* 12866-72, 1991.

13.	Liu, X. H. and Rose, D. P. Differential expression and regulation of cyclooxygenase-1 and -2 in two human breast cancer cell lines, Cancer Research. *56:* 5125-7, 1996.

14.	Rolland, P. H., Martin, P. M., Jacquemier, J., Rolland, A. M., and Toga, M. Prostaglandin in human breast cancer: Evidence suggesting that an elevated prostaglandin production is a marker of high metastatic potential for neoplastic cells, Journal of the National Cancer Institute. *64:* 1061-70, 1980.

15.   Futaki, N., Takahashi, S., Yokoyama, M., Arai, I., Higuchi, S., and Otomo, S. NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro, Prostaglandins. *47:* 55-9, 1994.

16.   Masferrer, J. L., Zweifel, B. S., Manning, P. T., Hauser, S. D., Leahy, K. M., Smith, W. G., Isakson, P. C., and Seibert, K. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic, Proceedings of the National Academy of Sciences of the United States of America. *91:* 3228-32, 1994.

17.   Sheng, H., Shao, J., Kirkland, S. C., Isakson, P., Coffey, R. J., Morrow, J., Beauchamp, R. D., and DuBois, R. N. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2, Journal of Clinical Investigation. *99:* 2254-9, 1997.

18.   Ethier, S. P., Mahacek, M. L., Gullick, W. J., Frank, T. S., and Weber, B. L. Differential isolation of normal luminal mammary epithelial cells and brest cancer cells from primary and metaststic sites using selctive media., Cancer Res. *53:* 627-635, 1993.

19.   Boxhorn, H. K. E., Smith, J. G., Chang, Y., Guerry, D., Lee, W. M. F., Rodeck, U., Turka, L. A., and Eck, S. L. Adenoviral transduction of melanoma cells with B7-1: anti-tumor immunity and immunosuppressive factors, Cancer Immunology Immunotherapy in press, 1998.

20.   Engelhardt, J. F., Yang, Y., Stratford-Perricaudet, L. D., Allen, E. D., Kozarsky, K., Perricaudet, M., Yankaskas, J. R., and Wilson, J. M. Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenoviruses, Nature Genetics. *4:* 27-34, 1993.

21.   Roberts, A. B. and Sporn, M. B. The transforming-growth-βs., p. 419-472. Heidelberg: Springer-Verlag, 1990.

22.   Strasnick, B., Lagos, N., Lichtenstein, A., and Mickel, R. A. First place--Resident Clinical Science Award 1990. Suppression of lymphokine-activated killer cell cytotoxicity by a soluble factor produced by squamous tumors of the head and neck, Otolaryngology - Head & Neck Surgery. *103:* 537-49, 1990.

23.   O'Mahoney, A., O'Sillivan, G., O'Connell, J., Cotter, T., and Collins, J. An immune suppressive factor derived from esophageal squamous cell carcinoma induces apoptosis in normal and transformed cells of lymphoid lineage, Journal of Immunology. *151:* 4847-4856, 1993.